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(54) Title: INFANT FEED

(57) Abstract

An infant feed comprises between about 1.6 g and 2 g protein per 100 ml and at least about 55 mg of calcium per 100 ml. The protein has a whey to casein ratio of greater than 1:1. The feed is used to nourish low birthweight infants which have reached a weight of at least about 1800 g.

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# INFANT FEED

The present invention relates to an infant feed and, in particular, to a feed with a high whey-predominant protein content which is suitable for feeding low birthweight infants, particularly premature infants, which have reached a weight of at least about 1800 g. The invention also provides a method and a use of such a feed for the nourishment of such infants.

The term 'low-birthweight' denotes an infant born at a weight of less than about 2500 g. Such infants are normally (but not necessarily) premature infants, that is infants born before the end of the normal nine months gestation period.

The expression 'term' denotes an infant aged at least nine months post-conception, whilst the expression 'pre-term' denotes a premature infant below this age, i.e. normally less than about 36 weeks post-conception.

A great many different infant feeds are known in the art such as, for example, the feeds disclosed in Dictionnaire Vidal, 1974, pages 910-911 and 913-914 and in Dictionnaire Vidal, 1978, pages 44-45.

A significant trend in the art has been the tendency to mimic the nutrient composition of natural human breast milk. It is a commonly accepted view that, in general, such a 'naturalised' formulation must be most suitable for normal infants. For example, in a Report of the Working Party on the Composition of Feeds for Infants and Young Children, Committee on Medical Aspects of Food Policy (DHSS Report No. 18) item 315, it is stated that "Although human milk, because of its variability, cannot be used as an exact chemical model for the composition of an infant feed, the Working Party

is of the opinion that human milk does provide the most useful reference 'standard'. The further the composition of any artificial feed departs from that of average mature human milk, the greater is the possibility of untoward effects in the infant to whom it is fed. It is important to ensure that infants are safeguarded from unsuitable feeds."

Typically, such humanised milk formulations contain about 1.5 g protein per 100 ml. For example, Dedicova and Drbohlov, in Veda-a-Vyzkum-v-Potravinarskem-Prungslu, 30, 127-142, describe 'humanised' infant feeds comprising variously 1.57 and 1.55 g protein per 100 ml.

In recent years it has been recognised that the feeding requirements in the first few weeks after birth of low birthweight infants, particularly very immature preterm infants, are specialised and not necessarily satisfied by feeds prepared with the needs of normal birthweight infants in mind.

EP-A-129418 (Farley Health Products) discloses an infant feed comprising inter alia 2 g protein and 70 mg calcium per 100 ml of feed. The formulation is stated to be intended primarily for use in the nourishment of infants have a body weight of 1850 g or less, more particularly 1200 g or less.

US-A-4216236 (Müeller et al) discloses a feed comprising inter alia 2.16 g whey-predominant protein and 52.5 mg calcium (per 100 ml) for use in the nourishment of new-born babies which are delicate, dysmature or premature.

Cow and Gate have marketed a low birthweight formula infant feed comprising inter alia 2.2 g protein and 108 mg calcium per 100 ml.

SMA have marketed a low birthweight formula infant feed comprising inter alia 2 g protein and 77 mg calcium per 100 ml.

Milupa have marketed a low birthweight formula infant feed comprising inter alia 2 g protein and 70 mg calcium per 100 ml.

The European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) has published guidelines for such feeds and recommends that they have an energy content of 65 to 85 kcal/100 ml, a protein content of 1.8 to 2.5 g/100 ml, a calcium content of 56 to 112 mg/100 ml, a copper content of 72 to 96 µg/100 ml, a zinc content of 440 to 880 µg/100 ml and a riboflavin content of 48 to 480 µg/100 ml. Such feeds are referred to herein as "pre-term formulae".

However, such formulations have normally been used only as in the early part of the infant's life, normally only until the end of hospitalisation. For example, it is stated at page 7, lines 33/36 of EP-A-0129418 that "... the infant foods according to the invention are especially suitable for the nourishment of very low birthweight infants, for example, such infants having a birthweight of 1850 g or less and more particularly a birthweight of 1200 g or less".

Use of enriched, particularly protein enriched formulations has been considered undesirable for longer periods (i.e. as so called 'term formulae') because, for example, it is known that protein makes a particularly significant contribution to the renal osmolar load, especially in the premature infant's renal function, and because it is known that proteins can elicit an allergic response in such infants.

Thus, where higher quality protein, such as whey-predominant protein, is used, the art teaches that levels should generally be kept low. For example, Lindblad et al, in Acta Paediatr. Scand, (1978), Vol 167 (5) pp 659-663, state that "a formula based on cow-milk protein should optimally contain only 1.0 to 1.2 g protein per 100 ml provided that it is 'humanised' not only with regard to the lactalbumin/casein ratio, but also the cystine and taurine content."

Indeed, some commentators have suggested that infants may down-regulate feed intake in relation to the energy or nutrient density, and that adding higher levels of protein may not therefore result in increased nutrient uptake (see, for example, Brookes and Kinsey, Arch. Dis. Child. (1985) 60, 42-46 and Foman et al, Acta Paediatr. Scand. (1975) 64, 172-181).

Levels of calcium are normally kept at 45 to 50 mg/100 ml, so as to guard against the risk of neonatal tetany. Greer, in J Nutrition, 1989, Vol 119 (12 Suppl) states that "The available information does not favour either increasing or decreasing the present concentrations of calcium, phosphorus or magnesium in infant formulas. The upper limit for these minerals should remain at the present concentrations: 45 to 50 mg/dl for calcium, 30-40 mg/dl for phosphorus...".

It has now surprisingly been found that, contrary to established thinking, low birthweight babies are not only able to tolerate higher levels both of better quality protein and of calcium in their feed, but that significantly improved development can be achieved in these infants by using feed which is enhanced in these components over a prolonged period following discharge from hospital.

According to the present invention, there is provided an infant feed comprising between about 1.6 and 2 g of protein per 100 ml, said protein having a whey to casein ratio of greater than 1:1, and at least about 55 mg of calcium per 100 ml.

Preferably, the feed comprises between about 1.7 and 1.9 g suitably about 1.85 g protein per 100 ml, and preferably between about 60 and 90 mg, suitably about 70 mg calcium per 100 ml. Preferably the protein has a whey to casein ratio of between about 55:45 and 65:35, suitably about 60:40.

Suitably, the feed comprises at least 100 µg, preferably about 650 µg of iron per 100 ml. Preferably, the feed comprises between about 20 and 50 mg, suitably about 35 mg phosphorus per 100 ml.

In an embodiment, the feed comprises between about 400 µg and 1 mg, suitably about 600 µg of zinc per 100 ml.

Preferably, the feed further comprises at least 3.5 µg, preferably about 5 µg of manganese per 100 ml and preferably at least 70 mg, preferably about 78 mg of potassium per 100 ml.

Preferably, the feed comprises between about 45 and about 110 µg, suitably about 60 µg copper per 100 ml.

Preferably, the feed is vitamin enhanced, with between about 40 and 100 µg, suitably about 95 µg of vitamin B1; between about 60 and 180 µg, suitably about 100 µg of vitamin B2; between about 40 and 100 µg, preferably about 80 µg of vitamin B6; between about 0.15 and 0.25 µg, preferably about 0.2 µg vitamin B12; between about 0.5 and 2 µg, preferably about 1.1 µg biotin; between about 3.4 and 50 µg, preferably about

25 µg folate; between about 690 and 1200 µg, suitably about 1000 µg niacin; between about 300 and 500 µg, preferably about 400 µg pantothenic acid; between about 7 and 28 mg, preferably about 15 mg vitamin C; between about 0.5 and 8 µg, preferably about 1.3 µg vitamin D, between about 0.5 and 10 mg, preferably about 1.5 mg vitamin E; and between about 3 and 7 µg, preferably about 6 µg vitamin K (all per 100 ml).

The infant feed will normally comprise fats and carbohydrates.

The fat content provides a proportion of the energy requirement, supplies essential fatty acids (EFAs) and aids the absorption of fat-soluble vitamins. Fat utilisation is less efficient in low birthweight infants because of immature digestive processes. Although human milk fat is relatively well absorbed, it is very difficult in practice to simulate using available sources of edible fats and oils. Fat digestion and absorption improve as fatty acid chain length decreases, the degree of unsaturation increases and the proportion of long chain saturated fatty acids such as palmitic acid in the outer positions of the triglyceride molecule diminishes. On physiological grounds, it is preferable to replace poorly absorbed long chain saturated fatty acids with a combination of mono- and poly-unsaturated fatty acids as in human breast milk and it has been found that this can be done in such a way as to ensure good fat absorption without providing excessive imbalances of individual fatty acids.

The carbohydrate content makes an important contribution to the energy requirement, the preferred carbohydrate for use being lactose. The infant feeds according to the invention also preferably contain a small amount (relative to lactose) of a glucose donor such as maltodextrin. It has been found that, for

example, maltodextrin improves the tolerance of the infant food by helping to make the osmolarity similar to body fluids and by increasing the margin of tolerance for lactose. The ratio of lactose:maltodextrin is with advantage from 5:1 to 9:1 by weight, advantageously about 6:1 by weight.

The invention also provides a method of feeding a low-birthweight infant by use of an infant feed as defined above. Preferably the feed is supplied only after the infant has reached a weight of about 1800 g, preferably about 2000 g, suitably about 2500 g. The feed will normally be supplied to an infant only after it is considered fit for discharge from hospital.

Beneficially, the feed is then supplied for a period of at least three months, preferably six to nine months, and in a preferred method the feed is supplied as the main source of nutrition until the infant is weaned and then as a dietary supplement throughout infancy.

According to a further aspect of the invention, there is provided the use of an infant feed as defined above to feed a low birthweight infant, and especially a premature infant, which has reached a weight of at least about 1800 g, preferably about 2000 g.

There is also provided the use of such a feed in the manufacture of an infant food to feed a term low-birthweight infant, and especially a premature infant, having a weight of at least about 1800 g, preferably about 2000 g.

The infant feeds according to the invention may conveniently be in ready-to-use, sterilised liquid form, in the form of a reconstitutable liquid concentrate or in solid (e.g. powder) form.

Suitable forms and methods for their production are described in European Patent Publication No. 0129418 in the name of the Boots Company PLC, which is herein incorporated by reference.

The invention is illustrated by the following example.

An infant feed (**Formula 1**) in accordance with the present invention was prepared to the following formula:

| | **Nutrient content** | |
|---|---|---|
| **Nutrient** | **per 100 ml** | **per 100K Cal** |
| Energy | 72 K cal | 100 K cal |
| Protein | * 1.85 g | 2.6 g |
| Fat | 4.0 g | 5.5 g |
| Carbohydrate | 7.2 g | 10.0 g |
| Calcium | 70 mg | 97 mg |
| Chloride | 45 mg | 62 mg |
| Copper | 57 µg | 80 µg |
| Iodine | 4.5 µg | 6.2 µg |
| Iron | 650 µg | 890 µg |
| Magnesium | 5.2 mg | 7.2 mg |
| Manganese | 5.0 µg | 7.0 µg |
| Phosphorus | 35 mg | 49 mg |
| Potassium | 78 mg | 110 mg |
| Sodium | 22 mg | 31 mg |
| Zinc | 600 µg | 840 µg |
| Vitamin A | 100 µg | 140 µg |

| | Nutrient content | |
|---|---|---|
| **Nutrient** | **per 100 ml** | **per 100K Cal** |
| Vitamin B1 | 95 µg | 130 µg |
| Vitamin B2 | 100 µg | 140 µg |
| Vitamin B6 | 80 µg | 110 µg |
| Vitamin B12 | 0.20 µg | 0.27 µg |
| Biotin | 1.1 µg | 1.5 µg |
| Folate | 25 µg | 35 µg |
| Niacin | 1000 µg | 1400 µg |
| Pantothenate | 400 µg | 560 µg |
| Vitamin C | 15 mg | 21 mg |
| Vitamin D | 1.3 µg | 1.8 µg |
| Vitamin E | 1.5 mg | 2.1 mg |
| Vitamin K | 6.0 µg | 8.4 µg |
| Taurine | 5.1 mg | 7.0 mg |
| Carnitine | 1.1 mg | 1.5 mg |
| Inositol | 3.2 mg | 4.4 mg |
| Choline | 5.1 mg | 7.0 mg |

(* denotes casein:whey ratio of 40:60)

This formula was compared with a conventional 'humanised' milk formulation (**Formula 2**) prepared to the following formula:

| | Nutrient content | |
|---|---|---|
| **Nutrient** | **per 100 ml** | **per 100K Cal** |
| Energy | 68 K cal | 100 K cal |
| Protein | * 1.45 g | 2.14 g |
| Fat | 3.82 g | 5.61 g |
| Carbohydrate | 6.96 g | 10.2 g |
| Calcium | 35 mg | 52 mg |
| Chloride | 45 mg | 67 mg |
| Copper | 42 µg | 61 µg |
| Iodine | 4.5 µg | 6.7 µg |
| Iron | 650 µg | 950 µg |
| Magnesium | 5.2 mg | 7.6 mg |
| Manganese | 3.4 µg | 5.0 µg |
| Phosphorus | 29 mg | 42 mg |
| Potassium | 57 mg | 84 mg |
| Sodium | 19 mg | 29 mg |
| Zinc | 340 µg | 500 µg |
| Vitamin A | 100 µg | 150 µg |
| Vitamin B1 | 42 µg | 61 µg |
| Vitamin B2 | 55 µg | 80 µg |
| Vitamin B6 | 35 µg | 52 µg |
| Vitamin B12 | 0.14 µg | 0.21 µg |
| Biotin | 1.0 µg | 1.5 µg |
| Folate | 3.4 µg | 5 µg |
| Niacin | 690 µg | 1000 µg |
| Pantothenate | 230 µg | 340 µg |
| Vitamin C | 6.9 mg | 10 mg |
| Vitamin D | 1.0 µg | 1.5 µg |
| Vitamin E | 0.48 mg | 0.71 mg |
| Vitamin K | 2.7 µg | 4.0 µg |
| Taurine | 5.0 mg | 7.4 mg |
| Choline | 4 8 mg | 7.0 mg |

These formulations were compared as follows:

32 pre-term infants were recruited after they had received neonatal intensive care. Each infant weighed less than 1850 g at birth. The criteria for inclusion were that they had been formula fed rather than breast milk fed during hospital stay, were free from congenital malformations and diseases likely to influence growth and neurodevelopment, weighed less than 3 kg at the time of entry to the study, and were aged less than 100 days. Infants were randomised to receive either the formula according to the invention (formula 1) or the comparative formula (formula 2) both in ready-to-feed form. Prior to randomisation, all the infants had been in a hospital intensive care unit, and had intensive data collection undertaken from birth.

The mean gestation, weights and post-natal ages at starting and the anthropometric data are given in Table 1. Data are means (with standard deviation [SD] shown in brackets) unless otherwise indicated.

**TABLE 1**

| | **Formula 1** (n=16) | **Formula 2** (n=15) |
|---|---|---|
| Gestation | 30.7(1.7) | 31.7(1.9) |
| Birthweight | 1513(173) | 1436(227) |
| Males : Females | 7 : 9 | 8 : 7 |
| Ventilated (n) | | |
| >1 day | 8 | 6 |
| >7 days | 1 | 0 |
| Days on intravenous nutrition [(a)]:median (IQR)* | 5(3,10) | 4(2,8) |
| Postmenstrual age at trial entry : weeks | 37(2) | 37(2) |
| Anthropometry at trial entry: | | |

| | Formula 1 (n=16) | Formula 2 (n=15) |
|---|---|---|
| Weight (kg) | 2.401(343) | 2.383(221) |
| Length (cm) | 46.3(2.2) | 46.0(1.4) |
| Head circumference (cm) | 33.2(1.2) | 33.3(1.0) |
| Triceps skinfold (mm) | 4.7(1.1) | 5.0(1.1) |
| Subscapular skinfold (mm) | 4.8(1.1) | 4.5(0.6) |

(a) partial or complete.
* Interquartile range.

A research nurse examined each infant fortnightly to record anthropometry. Weight was measured to the nearest 10 g using a Sartorius MP (Trade Mark) electronic balance; length was measured using a horizontal stadiometer to the next succeeding 1 mm; occipitofrontal circumference was measured to the next succeeding 1 mm using a paper tape measure; skinfold thicknesses - triceps and subscapular - were measured to the nearest 0.1 mm using Harpenden calipers.

The infants received the assigned diet, either as a sole source of nutrition or in conjunction with other foods until 9 months corrected postnatal age.

Student's t-test and chi-squared analyses were used to compare the two groups at specific corrected postnatal ages.

Longitudinal growth performance was compared in the two groups by multiple linear regression analysis using successive attained anthropometric measurements as dependent variables with previous measurements and diet type as independent factors on a within-subject basis, using a quadratic fit.

Based on previous follow-up data on preterm infants, sample size was calculated to detect a 10% difference in weight gain to 9 months post term at 5% significance and 80% power.

The growth performance of the two diet groups are shown for both sexes plotted on combined centiles in Figures 1 to 3 (based on Gardner-Pearson growth charts). Figures 1 to 3 show longitudinal data (means + SE [standard error] for (a) body weight, (b) body length, (c) head circumference respectively, in babies fed a standard formula [formula 2] (solid line) versus those fed the follow-on formula [formula 1] (dotted line) from recruitment (mean 3 weeks pre-term) to 40 weeks post-term. Data are for both sexes combined; centiles derived from Gardner-Pearson Charts (published by Castlemead, UK). Differences between the diet groups are apparent on visual inspection of the chart. At 37 weeks post menstrual age, on entry to the study, body weight lay between the 3rd and 10th centiles. By 9 months this was still the case for infants fed on standard formula, but those fed the nutrient enriched formula lay close to the 25th centile. Body length at 37 weeks post menstrual age lay close to the 25th centile in both groups. This was still so at 9 months for the infants fed the standard formula but those fed the enriched formula remained close to the 50th centile from 4 months post-term onwards. Significant differences between feed groups in body weight and length were seen at some individual time periods. However, a within-subject analysis to examine longitudinal growth rate, using a quadratic fit, showed significant increases in weight gain ($p<0.005$) and linear grown ($p<0.01$) in the nutrient-enriched diet (formula 1) fed group compared with those in the standard formula (formula 2) fed group throughout the whole 9 month study period

Feed intake and weaning

Table 2 shows milk volume intake in litres at corrected postnatal ages.

During the periods 0-3 months and 3-6 months post-term formula intake was the same in the two groups. The trend towards reduced formula intake in the group fed the nutrient enriched milk at 6-9 months was not significant. In addition, there was no significant difference in the time of introduction of weaning foods: mean (SD) corrected age 10.7 (4.5) weeks in the formula 2 group and 12.6 (9.2) weeks in the formula 1 group.

**TABLE 2**

| | 0-3 months (mean, SD) | 3-6 months (mean, SD) | 6-9 months (mean, SD) |
|---|---|---|---|
| Formula 1 (n=16) | 71.6 (14.9) | 69.8 (12.2) | 65.6(23.9) |
| Formula 2 (n=15) | 73.4 (13.5) | 69.4 (15.4) | 75.1 (32.0) |

Feed tolerance.

Data were collected on the number of vomits, possets and bowel motions for each infant on a day-to-day basis, the number of episodes of colic, stool consistency and volume (using charts developed for studies in gastrointestinal upset in UK and Gambian children by the MRC Dunn Nutritional Unit). There was no difference between the diet groups for any of these factors, although individual variation was large. Data in respect of feed tolerance is shown in Table 3, and stool data is shown in Table 4. There was an overall

trend towards larger stool weights in infants receiving formula 1.

**TABLE 3**

| | Vomits/day (median, IQR) | Possets/day (median, IQR) | Colic episodes/ fortnight (median, IQR) |
|---|---|---|---|
| Formula 1 (n=16) | 0.6(0.2-1.3) | 2.3(1.0-5.5) | 1 (0-4) |
| Formula 2 (n=15) | 0.9(0.1-1.8) | 1.7(1.1-4.2) | 1 (0-3) |

**TABLE 4**

| | | Corrected age (months) | | |
|---|---|---|---|---|
| | | 0-3 | 3-6 | 6+ |
| Formula 1 (n=16) | No/day (SE) | 2.2(.24) | 1.9(.14) | 2.0(.31) |
| | Size*(SE) | 3.3(.14) | 3.4(.15) | 3.6(.17) |
| | Consistency+ (SE) | 2.8(.13) | 2.3(.16) | 2.5(.09) |
| Formula 2 (n=15) | No/day(SE) | 2.0(.18) | 1.5(.06) | 1.8(.08) |
| | Size(SE) | 3.1(.15) | 3.4(.07) | 3.3(.08) |
| | Consistency (SE) | 2.9(.11) | 2.5(.14) | 2.4(.07) |

* size graded using comparison charts

| | |
|---|---|
| 1 | approx 1 g |
| 2 | approx 2.5 g |
| 3 | approx 5 g |
| 4 | approx 10 g |
| 5 | approx 20 g |

+consistency graded using comparison

| | |
|---|---|
| 1 | Hard |
| | 2Formed soft |

| | | |
|---|---|---|
| charts | 3 | Mushy soft |
| | 4 | Runny |
| | 5 | Watery |

30 of the 32 infants recruited to the study were further monitored with respect to bone mineralisation at 3 and 9 months corrected age.

Measurement of bone width and mineral content was undertaken using single photon absorptiometry using equipment available under the trade designation "Lunar SP2" from Lunar Radiation Corporation. A collimated beam of photons from an $^{125}I$ source was passed across the arm to a photomultiplier detector. Source and detector were moved in tandem across the arm, and the bone width and mineral content were calculated in known manner from the attenuation of the photon beam. Each infant was placed supine with the left arm extended. The forearm was enfolded in a tissue-equivalent bag made from dialysis tubing filled with warm water. With the arm held perpendicular to the beam path, two scans across the forearm were undertaken along the same track. Where the difference between scans exceeded 5% the scan was repeated.

Measurements were undertaken at the one third distal site, that is the position corresponding to one third of the distance from the tip of the olecranon to the ulnar styloid process, measured distally from the styloid process. The radius at this point approximates a cylinder over a two or three centimetre distance and thus provides a geometrically stable measuring area.

The radial bone width (BW) and bone mineral content (BMC) estimations for each group before discharge from hospital and at 3 and 9 months corrected age are shown in table 5. There are significant differences in BMC at

both post-discharge time periods (t=3.53, p=0.001, t=3.15, p=0.004 at each age respectively).

**TABLE 5**

(i) Bone width (cm)

| Age | Formula 1 | Formula 2 | Difference between means (95% CI) |
|---|---|---|---|
| Before discharge | 0.368 (0.013) | 0.360 (0.010) | 0.008 (-0.023 - 0.039) |
| 3 months | 0.581 (0.021) | 0.565 (0.024) | 0.16 (-0.046 - 0.078) |
| 9 months | 0.669 (0.022) | 0.619 (0.022) | 0.050 (-0.011 - 0.111) |

(ii) bone mineral content (g/cm)

| Age | Formula 1 | Formula 2 | Difference between means (95% CI) |
|---|---|---|---|
| Before discharge | 0.035 (0.003) | 0.035 (0.003) | 0.000 (-0.008 - 0.008) |
| 3 months | 0.083 (0.004) | 0.063 (0.004) | 0.020 (0.009 - 0.032) |
| 9 months | 0.115 (0.005) | 0.095 (0.004) | 0.020 (0.007 - 0.033) |

It would be reasonable to expect that larger babies should have bigger bones and therefore more bone mineral. A graphical comparison of the performance of the infants in this study with that of infants born at term on the basis of corrected post-natal age is shown in figure 4. Comparison when the expected age for their body size is used is shown in figure 5. In each figure, the black squares represent infants receiving Formula 1,

the open circles represent infants receiving Formula 2, the topmost solid line represents term infants of both sexes, and the broken line represents term infants (3rd centile). It is clear that differences in body size alone do not account for the variation in bone mineral content. It is also apparent that the velocity of bone mineralisation in both groups exceeds that of the term infants.

Multiple regression analysis was undertaken to identify further factors likely to influence bone mineral content at 3 and 9 months corrected age. This analysis is shown in table 6 where "Diet" represents the effect of receiving the supplemented diet and "Sex" represents the effect of being male. Post-discharge diet was the factor most strongly associated with bone mineral content at 3 months ($t=4.3$, $p=0.0002$), exceeding the association of bone width ($t=3.2$, $p=0.004$) and the effect of being male ($t=2.62$, $p=0.014$). At 9 months, post-discharge diet was still independently associated with bone mineral content ($t=2.62$, $p=0.14$) although the effect of bone width was then much stronger ($t=4.59$, $p=0.0001$). The effect of diet at 9 months was reduced ($t=1.11$, $p=0.28$) if bone mineral content at age 3 months was included in the model.

**TABLE 6**

(i) 3 months

| Factor | Regression Coefficient | SE Coefficient | T value | P value |
|---|---|---|---|---|
| Diet | 0.020 | 0.005 | 4.34 | 0.0002 |
| Bone width (cm) | 0.087 | 0.027 | 3.21 | 0.0036 |
| Sex | 0.012 | 0.005 | 2.59 | 0.016 |

(ii) 9 months

| Factor | Regression Coefficient | SE Coefficient | T value | P value |
|---|---|---|---|---|
| Diet | 0.014 | 0.005 | 2.62 | 0.014 |
| Bone width (cm) | 0.140 | 0.030 | 4.59 | 0.0001 |
| Sex | 0.001 | 0.0001 | 0.54 | 0.59 |

The results show a significant difference in radial BMC between the groups by 3 months corrected post-natal age, persisting to age 9 months.

Bone mineral content estimated by SPA correlates well with total body calcium measured by in-vivo neutron activation analysis and dual energy X-ray absorptiometry in adults. The results therefore suggest an overall increase in the total amount of bone mineral in the skeleton for those infants who received Formula 1.

It is concluded that the formula of the present invention was well-tolerated by the infants which received it, and resulted in significant improvements in well-being.

Claims

1. An infant feed for feeding a low birthweight infant, comprising between about 1.6 and 2 g protein per 100 ml, said protein having a whey to casein ratio of greater than 1:1, and at least about 55 mg of calcium per 100 ml.

2. An infant feed as claimed in Claim 1 with further comprises between about 20 and 50 mg phosphorus per 100 ml.

3. An infant feed as claimed in Claim 1 or Claim 2 which comprises between about 1.7 and 1.9 g protein per 100 ml.

4. An infant feed as claimed in any one of the preceding claims which comprises between about 60 and 90 mg calcium per 100 ml.

5. An infant feed as claimed in any one of the preceding claims wherein the protein has a whey to casein ratio of about 60:40.

6. An infant feed as claimed in any one of the preceding claims which is enhanced in vitamin and minerals compared to normal human breast milk.

7. A method of nourishing a low birthweight infant by administering an infant feed as claimed in any one of the preceding claims when the infant has reached a weight of at least about 1800 g.

8. A method as claimed in Claim 7 wherein the infant is administered a pre-term formula infant feed having at least 2 g protein per 100 ml until the infant has reached a weight of up to about 1800 g.

9. The use of an infant feed as claimed in any one of claims 1 to 6 to feed a low birthweight infant.

10. The use of an infant feed as claimed in any one of claims 1 to 6 in the manufacture of a medicament to feed a low birthweight infant.

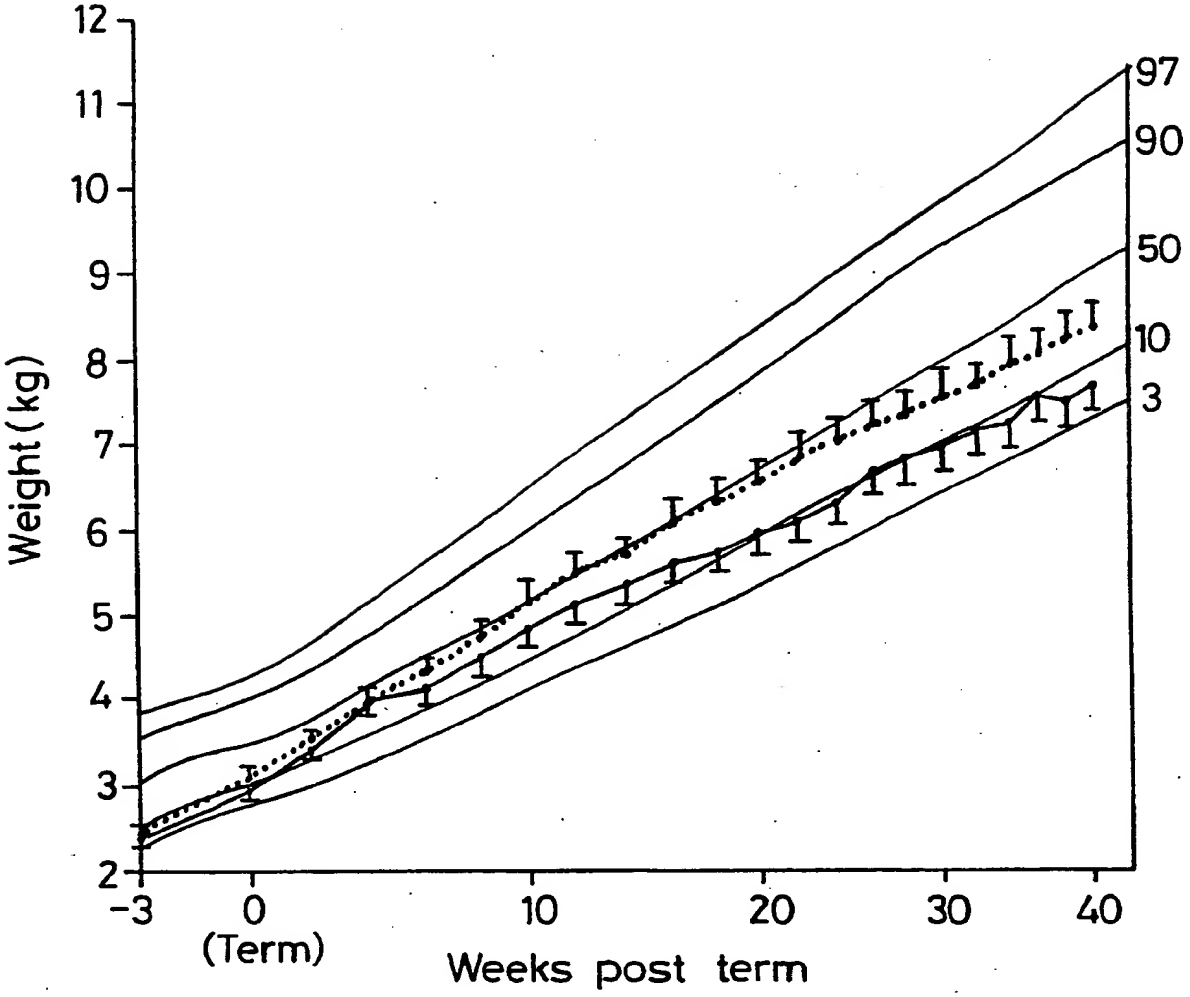
12
11
10
9
8
7
6
5
4
3
2
Weight (kg)
97
90
50
10
3
-3
0
(Term)
10
20
30
40
Weeks post term

Fig. 1

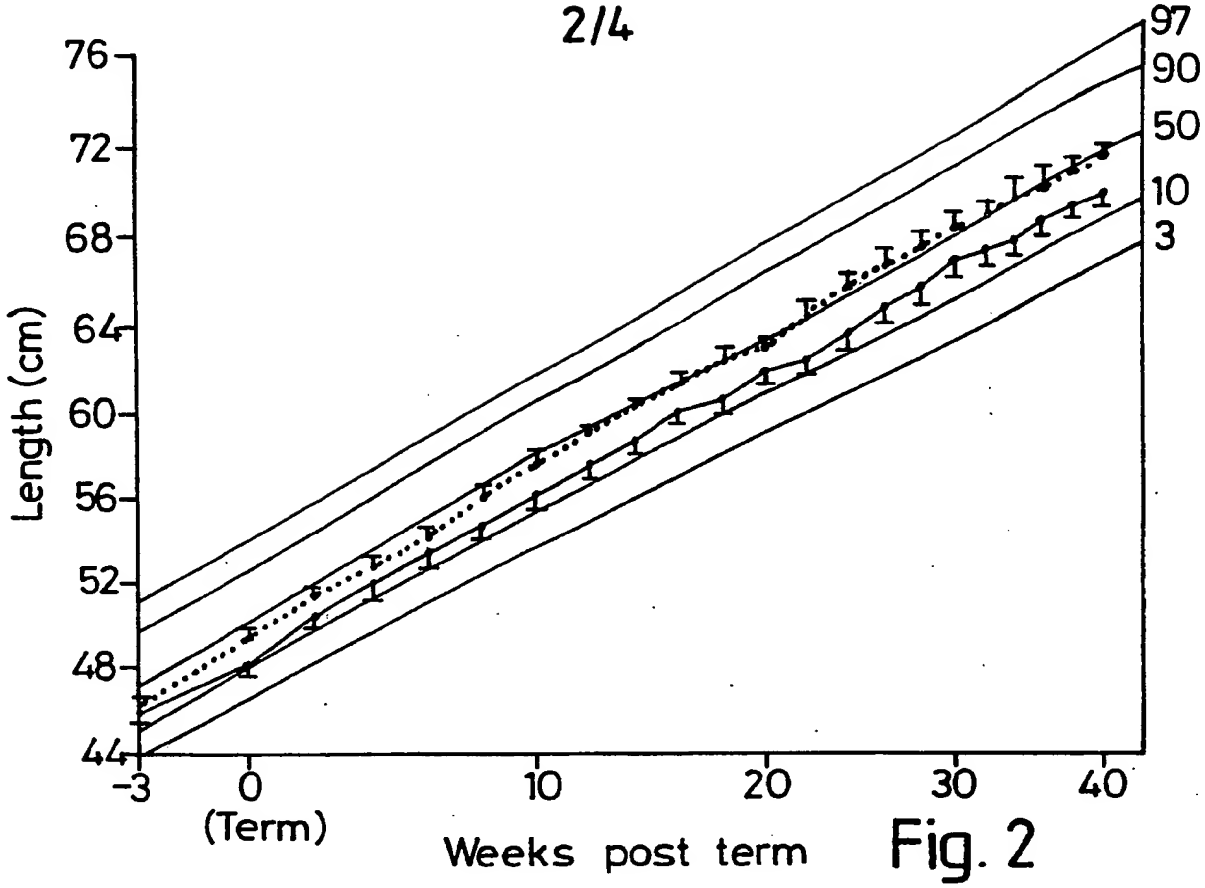
Length (cm)
76
72
68
64
60
56
52
48
44
97
90
50
10
3
-3
0
(Term)
10
20
30
40
Weeks post term

Fig. 2

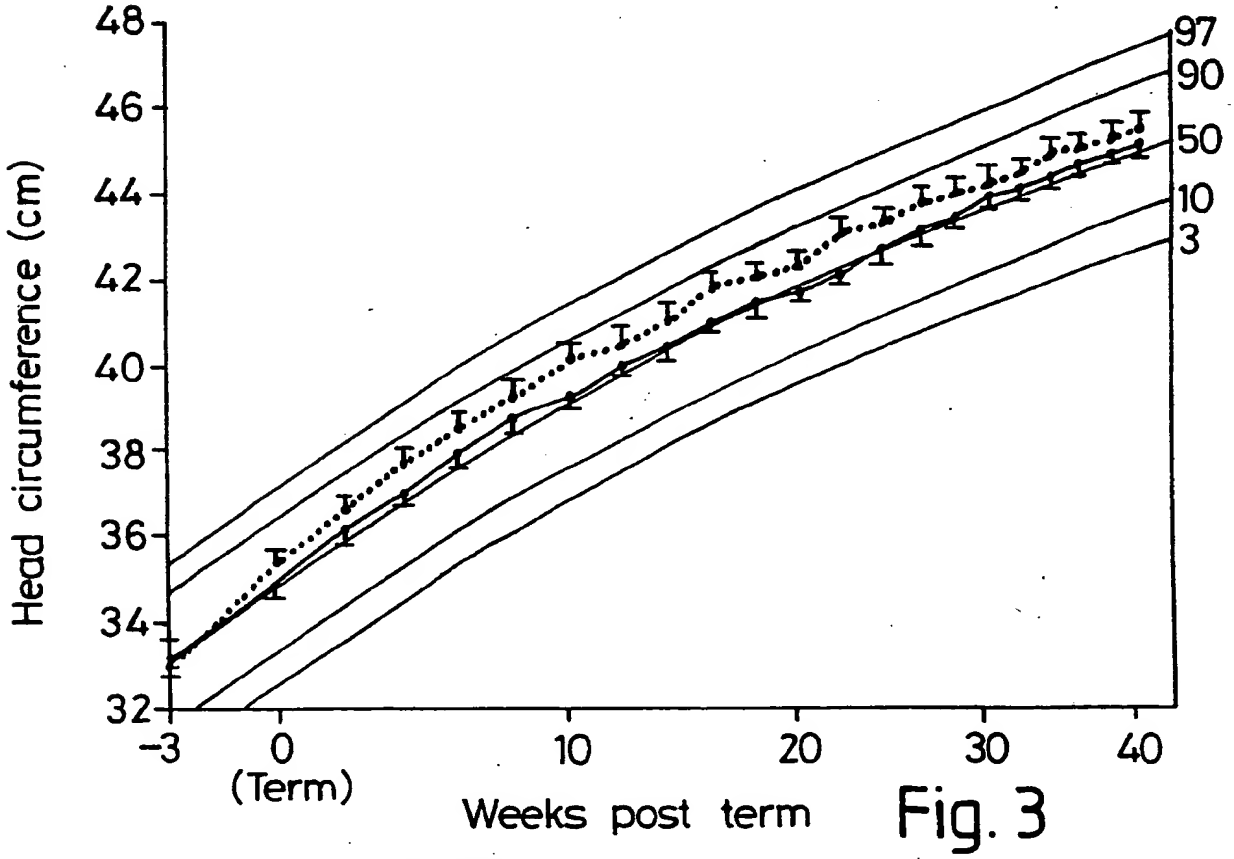
Head circumference (cm)
48
46
44
42
40
38
36
34
32
97
90
50
10
3
-3
0
(Term)
10
20
30
40
Weeks post term

Fig. 3

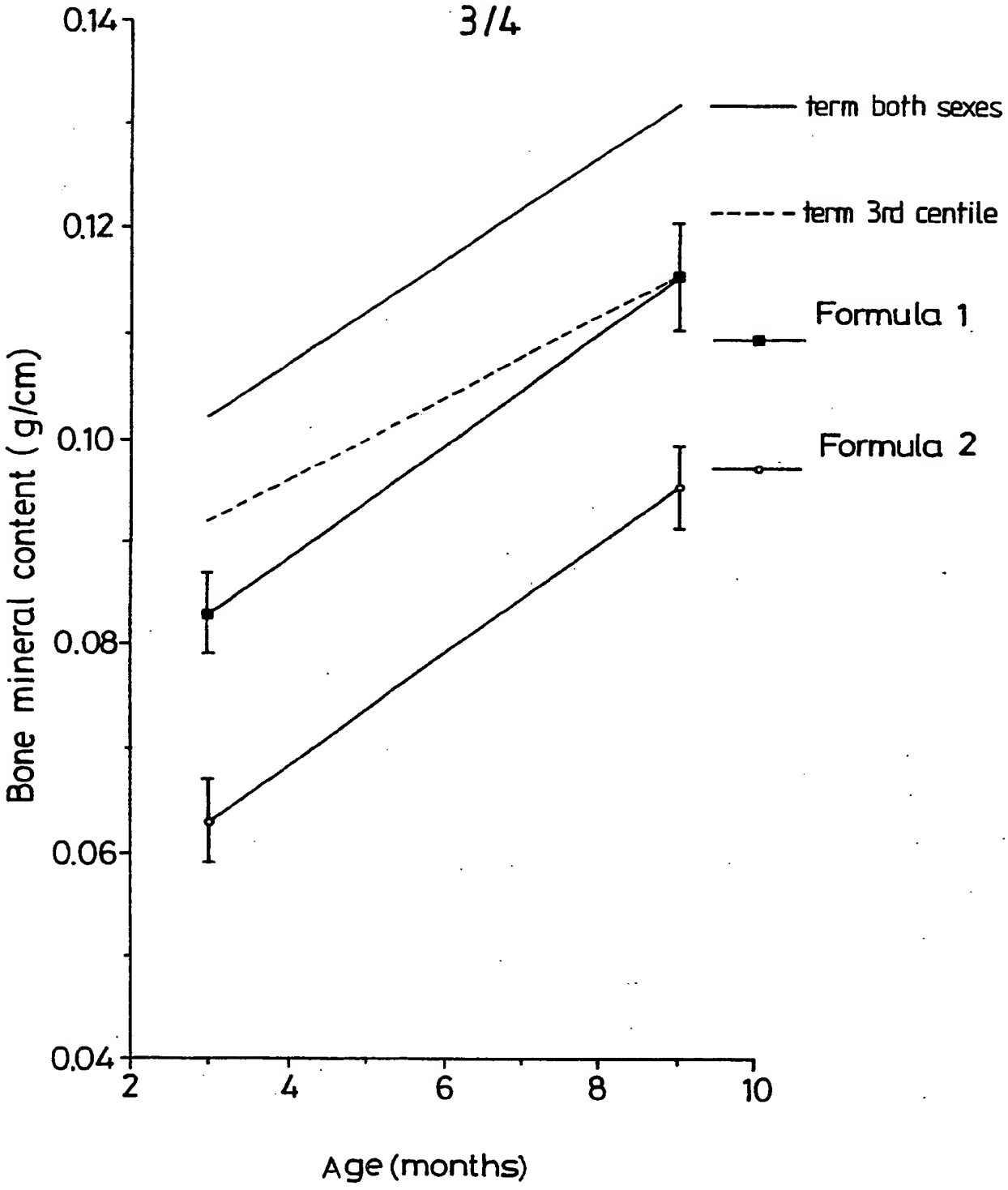
0.14
0.12
0.10
0.08
0.06
0.04
Bone mineral content (g/cm)
2
4
6
8
10
Age (months)
term both sexes
term 3rd centile
Formula 1
Formula 2

Fig. 4

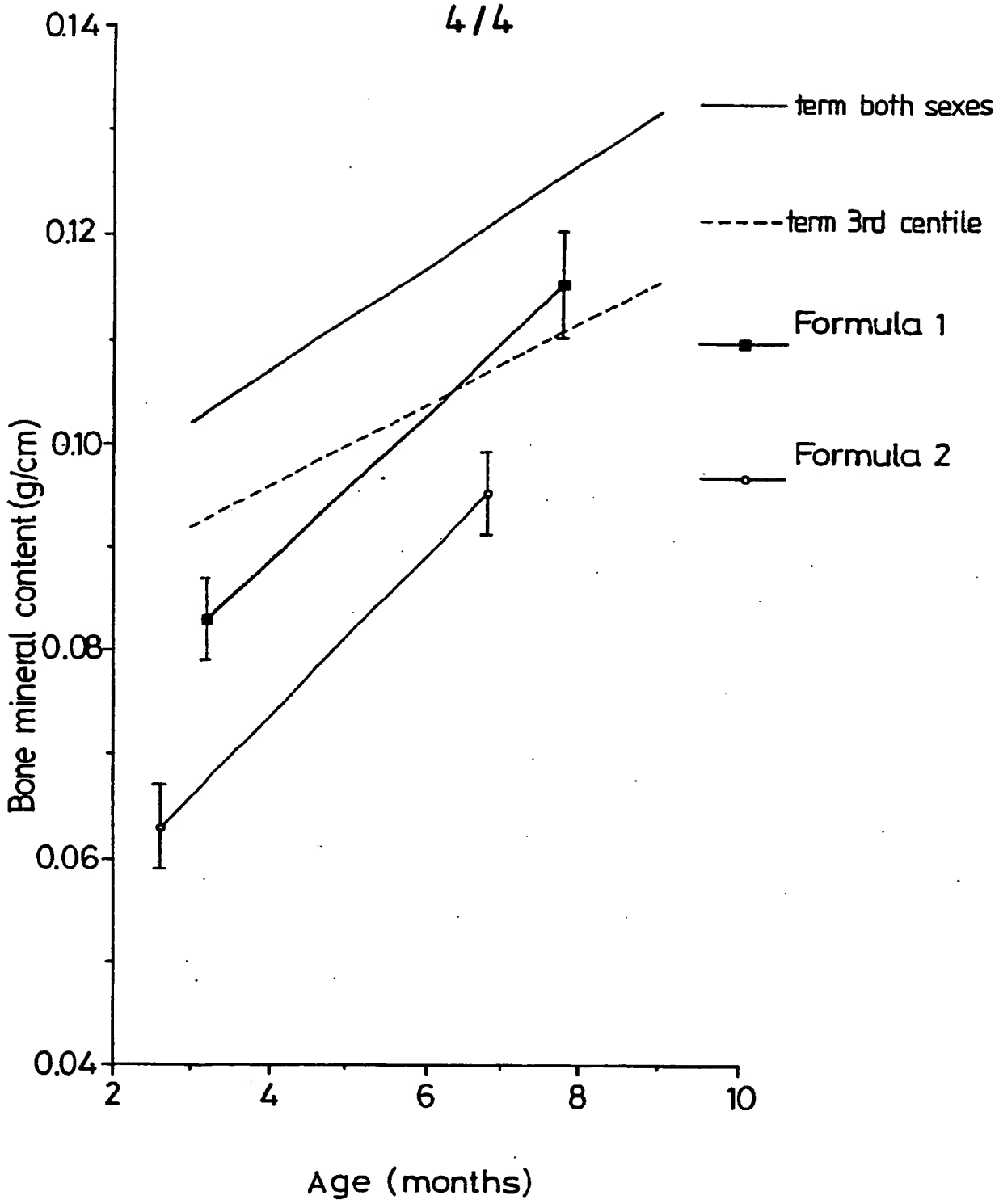
0.14
0.12
0.10
0.08
0.06
0.04
Bone mineral content (g/cm)
2
4
6
8
10
Age (months)
term both sexes
term 3rd centile
Formula 1
Formula 2

Fig. 5

# INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/00394

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A23C9/20; A23C9/15; A23L1/305

## II. FIELDS SEARCHED

Minimum Documentation Searched[7]

| Classification System | Classification Symbols |
|---|---|
| Int.Cl. 5 | A23C ; A23L |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

| Category ° | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No.[13] |
|---|---|---|
| X | EP,A,0 129 418 (GLAXO GROUP)<br>27 December 1984<br>cited in the application<br>see claims 1-15<br>see page 12, line 35 - page 13, line 20<br>see page 15, line 3 - page 16, line 18<br>see page 16, line 29 - page 17, line 2;<br>table 7 | 1-6,10 |
| A | FR,A,2 388 503 (NESTLÉ)<br>24 November 1978<br>see claims; example 1<br>& US,A,4 216 236<br>cited in the application | 1-6,10 |
| A | US,A,4 337 278 (R.A.BROG)<br>29 June 1982<br>see claim 1; example III | 1-6,10 |

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 11 JUNE 1993 | 11. 07 93 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUR PEAN PATENT OFFICE | VAN MOER A.M.J. |

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
|---|---|---|
| X | WO,A,9 108 675 (C.SLATERY)<br>27 June 1991<br>see page 17, line 11 - page 21, line 5;<br>claims 1,6 | 1-6,10 |
| A | GB,A,1 446 431 (J.WILLIAMS)<br>18 August 1976<br>see example VIII | 1-6,10 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 93/00394

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7,8,9:
because they relate to subject matter not required to be searched by this Authority, namely:
METHOD FRO TREATMENT OF THE HUMAN BODY BY THERAPY/

PLEASE SEE PCT RULE 39.1(iv)!!

2. [X] Claims Nos.: 6
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
MERELY STATING THAT AN INFANT FEED IS "ENHANCED IN VITAMINE AND MINERALS CO MPARED TO NORMAL HUMAN BREAST MILK" DOES NOT PERMIT TO KNOW WHAT VITAMINE(S ) AND/OR MINERALS ARE USED TO THIS AIM AND WHAT THEIR PROPORTIONS ARE.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9300394
SA 71809

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent ffice EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 11/06/93

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| | | GB-A- | 1581900 | 31-12-80 |
| | | NL-A- | 7804492 | 31-10-78 |
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| | | SE-A- | 7804725 | 28-10-78 |
| | | US-A- | 4216236 | 05-08-80 |
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| | | FR-A,B | 2197605 | 29-03-74 |